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Preventing Parkinson's pathology

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Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain. The impaired production and secretion of dopamine causes a variety of symptoms, including bradykinesia, tremor, rigidity, and other motor and cognitive problems. Although the disease was first described in 1817 (Parkinson, 2002), few treatments exist today. These treatments do not target the cause of the disease and instead aim to increase the levels of dopamine.

A major difficulty in understanding PD is the complexity of disease onset. Multiple pathways, including protein aggregation, defects in the ubiquitin-proteasome system, mitochondrial damage, and oxidative and nitrosative stress, can all contribute to the loss of dopaminergic neurons (Dawson and Dawson, 2003). How these pathways are activated and interconnected remains a question of the utmost importance.

The protein α-synuclein (α-syn) appears to play a key role in the pathogenesis of PD (Gitler and Shorter, 2007). α-Syn is the major component of Lewy bodies, the pathological hallmark of the disease, and mutations in the α-syn gene (*SNCA*) cause PD in rare familial forms of the disease. Additionally, duplication and triplication of wild-type *SNCA* has also been linked to PD.

Most model organisms of PD have been designed to express α-syn because of its prominent role in the disease. These biological tools, which have now expanded to yeast (Outeiro and Lindquist, 2003), *Caenorhabditis elegans* (van Ham et al., 2008), *Drosophila melanogaster* (Feany and Bender, 2000) and mammalian models (Masliah et al., 2000), help to define how pathways interact, establish early markers of the disease, and facilitate the discovery of drugs that may slow or prevent the pathology.

Models in yeast or any other organism cannot perfectly recapitulate the disease as seen in humans. However, models have proven to be useful in the past for uncovering key players in human diseases. Two examples of the success of the yeast α-syn model are the Rab GTPase Ypt1 (Cooper et al., 2006) and a putative manganese transporter, YPK9 (Gitler et al., 2009). These proteins were discovered in genetic screens for suppressors of α-syn toxicity and were validated in a variety of animal and cell culture models of PD (Cooper et al., 2006; Gitler et al., 2009). Indeed, the human homolog of YPK9, PARK9, was shown to be involved in the onset and pathology of PD (Gitler et al., 2009; Ramirez et al., 2006). Clearly, the yeast model system is a powerful tool for elucidating the molecular basis for disease pathways.

In a recent issue of *DMM*, Su et al. utilize a yeast strain expressing toxic levels of α-syn to examine transcriptional differences and to screen for small molecules that reduce toxicity (Su et al., 2009). The authors found that α-syn overexpression affected the transcriptional profile and morphology of yeast cells. Transcriptional characterization of the α-syn-expressing cells showed significant changes in transcription long before cell death had occurred. Genes involved in mitochondrial function and maintenance were downregulated, whereas oxidoreductase genes were upregulated (see fig. 1 in Su et al.). The morphological hallmarks of α-syn overexpression included lipid droplet accumulation, endoplasmic reticulum (ER)-Golgi trafficking defects, misshapen mitochondria, high levels of reactive oxygen species (ROS), and accumulation of cytoplasmic α-syn foci (see fig. 2 in Su et al.). These are significant findings because they connect the yeast model of α-syn aggregation with several key neuronal phenotypes of PD.

A major breakthrough described by this paper arose out of an extensive small molecule screen examining the effect of over 100,000 chemical compounds on α-syn toxicity in yeast. A group of four 1,2,3,4-tetrahydroquinolinone compounds [denoted (1), (2), (3) and (4)] emerged from the screen as suppressors of α-syn toxicity (see fig. 3A in Su et al.). In α-syn-expressing cells, the bioactive compounds prevented many transcriptional changes caused by α-syn expression. Importantly, the compounds ameliorated the symptoms of α-syn aggregation: ROS levels decreased, α-syn foci vanished, ER-Golgi trafficking resumed, and mitochondrial morphology was restored. The structurally related compounds (5) and (6) did not act as suppressors of α-syn toxicity, but did competitively inhibit the bioactive compounds, suggesting that all of the tetrahydroquinolinones tested bound to the same target(s).

Although they were unable to elucidate the exact mechanism of action of the bioactive molecules, the authors were able to rule out a few possibilities. First, the compounds were specific antagonists of α-syn toxicity because they did not affect cell viability in yeast carrying a toxic polyQ construct. This suggests that the compounds were not general modulators of the cellular stress responses caused by protein aggregation. Second, the bioactive molecules did not affect levels of α-syn in vivo, or aggregation of α-syn in vitro, which the authors interpreted as evidence that the compounds did not directly influence the expression of α-syn, or directly interact with α-syn, respectively. Finally, the authors showed that the compounds were not simply acting as free radical scavengers because the antioxidants N-acetylcysteine, riboflavin and melatonin did not alter α-syn toxicity in the yeast model.

To extend their findings and determine whether the α-syn toxicity suppressors from their yeast screen were efficacious in vivo, the authors tested compounds (1-6) in a nematode model of PD. The nematode *C. elegans* is an attractive model system because the expression of α-syn in its neurons results in an age-dependent loss of dopaminergic neurons, a phenotype that closely resembles that seen in humans (Cao et al., 2005). Compounds (1-4), but not compounds (5-6), were found to suppress α-syn toxicity. For the most bioactive compound, (1), this suppression resulted in a 20% increase in the number of worms with all four dopaminergic neurons. Remarkably, the bioactive compounds were also able to partially reverse the phenotype of

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worms that were expressing α-syn for two days prior to treatment (see fig. 4 in Su et al.). This observation is particularly exciting because it means that these compounds might hold promise for treating the disease once it is initiated, rather than having to treat before the onset of symptoms.

To determine whether their findings were relevant to mammals, the authors used embryonic rat midbrain cultures transduced with α-syn-A53T, a variant of α-syn linked to early-onset PD. Expression of the A53T mutant induced reproducible cell toxicity. Additionally, surviving neurons displayed aberrant morphology. The compounds (1-3) and (6) were potent suppressors of α-syn toxicity in these rat midbrain cultures, resulting in an increase in the relative number of dopaminergic neurons. Neuron morphology also remained normal. Compounds (4) and (5) did not affect α-syn toxicity (see fig. 4 in Su et al.). Interestingly, compounds (1) and (4), and to a lesser extent (3) and (5), prevented rotenone-induced toxicity (see fig. 6 in Su et al.). Rotenone is a mitochondrial complex I inhibitor that is often used to model PD-like mitochondrial damage in rodents. This finding is significant because it illustrates that the compounds can cure PD symptoms that are not directly caused by α-syn aggregation.

The results presented in this article are exciting; however, it is still unclear whether the bioactive molecules are truly effective at *reversing* aberrant cell phenotypes after the α-syn aggregation has caused cellular toxicity. Although rescue experiments were performed in *C. elegans* with positive results, it is necessary to perform these studies in other systems to show that the compounds are able to reverse the α-syn phenotype and not simply prevent it. Effective PD drugs need to be administered after the onset of disease, not before. Additionally, the cellular target of the bioactive compounds is unknown, and there is not enough evidence to conclude that the compounds are not interacting with α-syn itself. Understanding how these molecules work could reveal much about the etiology of PD. Affinity chromatography with immobilized bioactive compounds could be used to identify their in vivo targets. Targets might also be identified by using chemical-genetic profiling in yeast (Parsons et al., 2004; Parsons et al., 2006). In this technique, libraries of haploid deletion mutants would be screened with the drugs to identify hypersensitive strains. Comparisons with known chemical-genetic profiles and genetic interaction maps could then be used to elucidate possible target pathways and illuminate the mode of action of these compounds.

The initial causes of PD are not fully understood. Although α-syn can clearly play a role in the development of the disease, multiple pathways are intimately linked: oxidative damage, proteasomal impairment, α-syn aggregation, mitochondria dysfunction and ER-Golgi trafficking defects may all contribute to the death of dopaminergic neurons. Although Su et al. (Su et al., 2009) do not offer insight into all aspects of this complex network of disease pathology, their results are very encouraging and bring us one step closer to the possibility of treating various cellular symptoms of PD with a single drug. In a broader sense, the work of Su et al. implies the existence of a target that is common to multiple pathways implicated in PD. Although currently elusive, this target presents an ideal therapeutic site.

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